FORM PTO-1390 (REV 11-98) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 1012-102US

U.S APPLICATION NO (If known, see 37 CFR 1.5): 09/719909

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/01499	12 May 1999	29 June 1998

TITLE OF INVENTION: INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY

APPLICANT(S) FOR DO/EO/US: Thomas William Rademacher, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Unsigned)
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

    Copy of IPER
    Marked Copy of Amended Claims
    Courtesy Copy of Claims as Amended
    Certificate of Mailing
    Acknowledgement Postcard

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/719909	PCT/GB99/01499	1012-102US

17. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$760.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	19 - 20 =	0	X $18.00	$ 0.00	
Independent claims	2 - 3 =	0	X $78.00	$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ 270.00	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1240.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 620.00	
SUBTOTAL =				$ 620.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ]20 [ ]30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 620.00	
				Amount to be: refunded	$
				charged	$ 620.00

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 50-0893 in the amount of $620.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0893. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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41,261
REGISTRATION NUMBER

Attorney Docket No. 1012-102US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Unassigned
Thomas William Rademacher, et al.	Art Unit: Unassigned
Application No.: Not yet known	
Filed: Herewith	PRELIMINARY AMENDMENT
For: INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

IN THE CLAIMS

Please amend the claims to as follows, without prejudice to subsequent renewal of the specification in its original form. **Per the requirements of 37 C.F.R. § 1.121, the following claims are to be substituted for the corresponding previously pending claims of the same number(s). A marked up version showing the changes to the claims, is attached herewith. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith.**

4. (Amended). The composition of claim 1, further comprising adenosine or purine, or a nucleotide precursor thereof.

7. (Amended). A composition of claim 1, for use in a method of medical treatment.

11. (Amended). The use of claim 8, wherein the ischaemic-reperfusion injury arises from myocardial infarct, surgery or stroke.

13. (Amended). The use of claim 8, wherein the medicament is for the prevention of apoptosis following an ischaemic-reperfusion injury.

14. (Amended). The use of claim 8 wherein the medicament further comprises one or more of:

(a) adenosine or purine or a precursor thereof;

(b) ribose;

(c) nicotinamide or derivatives thereof;

(d) a Ca^{2+} ion uptake inhibitor;

(e) a cardioplegic solution;

(f) means to maintain the glutathione system, such as glutathione peroxidase and the reduced form of glutathione (GSH); or,

(g) an endothelin inhibitor.

15. (Amended). An in vitro method for preserving an organ for transplantation, the method comprising contacting the organ with a composition of claim 1.

REMARKS

The Applicant has made corrections to claims in order to conform to U.S. practice and no new matter is introduced by the amendment

CONCLUSION

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 510-337-7871.

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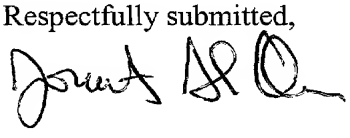
Respectfully submitted,



Jonathan Alan Quine, J.D., Ph.D.
Reg. No. 41.261

INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY

Field of the Invention

The present invention relates to material and methods relating to the prevention or treatment of ischaemia-reperfusion injury, and in particular to compositions comprising inositolphosphoglycans (IPGs) and their medical use in the prevention or treatment of ischaemia.

Background of the Invention

The search for novel therapies for ischaemic-reperfusion injury in the heart has been a subject of intense research, both for recovery from open-heart surgery, where the limited capacity for the heart to survive ischaemia is a well researched problem (Stanley et al, 1997), and from the viewpoint of modulating the extent of damage incurred during episodes of cardiac ischaemia (Stanley et al, 1997). It is also well established that the incidence of coronary heart disease is a major factor in the morbidity and mortality of diabetic patients (Fuller et al, 1983; Hillier et al, 1988). There is also evidence that standard drugs for the treatment of diabetes of the sulphonylurea group may have negative effects, including those on K^+ channel function (Smits & Thien, 1995; Muhlhauser et al, 1997).

The complexity of the events following ischaemia-reperfusion is such that there is a very wide ranging database of potential therapeutic and cardioplegic agents targeting differing aspects of the cascade leading to damage to cardiac function. It has been apparent from

work as early as the 1960s (Danforth et al, 1960; Berne, 1963) to the present (Zimmer, 1996; Houston et al, 1997) that a key feature of the cascade of interlinked biochemical events following ischaemic-reperfusion injury centres on the loss of adenine nucleotides from the myocardium. There is, thus, an absolute requirement for the restitution of the intracellular ATP concentration and the energy charge of the cell in order to restore normal cardiac function.

Adenine nucleotide synthesis can occur via utilization or reutilisation of adenine nucleotide breakdown products via the salvage pathway, or via *de novo* synthesis from small molecular weight precursors. The former is the most effective in terms of energy requirement (Mangano, 1997; Meldrum et al, 1997).

However, in addition to the requirement for the purine ring, a supply of phosphoribosylpyrophosphate (PRPP) is essential both for the salvage and *de novo* routes of synthesis; this latter compound is, in turn, subject to tight regulation and is dependent upon a supply of ribose-5-phosphate (Kunjara et al, 1987). Zimmer (1980) demonstrated that restitution of myocardial adenine nucleotides was accelerated by ribose, as was the normalisation of depressed heart function in rats (Zimmer, 1983). This author stated that "The advantage of ribose over other metabolic interventions is that is does not affect the haemodynamics of the heart with an ultimate change in oxygen demand and that is has no vasoactive properties which may result in afterload

alterations".

Recently, Zimmer (1996) reported that in two *in vivo* rat models, the overloaded and catecholamine-stimulated heart and the infarcted heart, the normalisation of the cardiac adenine nucleotide pool by ribose was accompanied by improvement in global heart function. Further, the combined treatment with ribose and adenine or inosine in isoproteronal-treated rats was more effective in the restoration and completely restored the ATP level within a shorter period of time than either treatment alone.

Summary of the Invention

While the results showing the effect of repletion of cardiac ATP are encouraging, the prior art approaches described above suffer from the disadvantage that the biosynthetic pathways themselves require ATP, as does the reconversion of AMP to ADP and ATP, the required ATP being the very compound in short supply. Further, as mentioned above, the complexity of the biochemistry associated with ischaemia means that it is not clear from the prior art how alternative approaches could avoid this problem.

The present invention relates to the finding that inositolphosphoglycans (IPGs), and in particular P-type IPGs, or their synthetic analogues, can be used to generate ATP from ADP while helping to avoid the production of toxic byproducts and helping to minimise the ATP requirement for the process. Thus, compositions comprising IPGs can be used to prevent or treat

ischaemia-reperfusion, in particular in conditions where there is a reduction or risk of reduction in cellular ATP levels, e.g. in cardiac ischaemia, in surgery (especially heart or transplant surgery), in preserving organs for transplantation, in the treatment of stroke and as an anti-apoptosis agent to protect against cell death (especially in muscle cells).

Accordingly, in a first aspect, the present invention provides a composition for treating an ischaemic-reperfusion injury, the composition comprising an inositolphosphoglycan (IPG) or an IPG synthetic analogue, and ribose

In a further aspect, the present invention provides the use of an inositolphosphoglycan (IPG) for the preparation of a medicament for the treatment of ischaemic-reperfusion injury.

The IPGs present in the medicament can be P- or A-type IPGs, or synthetic analogues of them. The production of IPGs and IPG analogues is discussed further below. Preferably, the IPG is a P-type IPG or a P-type synthetic analogue.

The present invention is based on the realisation that an alternative approach to the problem of increasing the energy generating systems of the cell is to employ the mitochondrial oxidative restoration system, in particular by the regulation of the key enzyme for the entry of pyruvate into the tricarboxylic acid cycle, pyruvate

dehydrogenase. Accordingly, the present proposal centres upon the use of naturally occurring activators of pyruvate dehydrogenase phosphatase, the inositolphosphoglycans, to promote the conversion of pyruvate dehydrogenase to the active form, thereby enhancing the rephosphorylation of AMP and ADP.

Advantageously, the composition includes one or more other components, in combination with the IPGs, for use in the treatment of ischaemia-reperfusion injury as described herein. Among the agents to be used in combination with IPGs from different sources are:

(1) Adenosine and purine compounds as precursors of ATP and as modulators of TNFα action (see Bouchard & Lamontagne, 1998; de Jong et al, 1997; Meldrum et al, 1997).

(2) Ribose as a precursor of PRPP (see Kunjara et al, 1987; Zimmer, 1996).

(3) Nicotinamide and derivatives to prevent the loss of NAD and ATP by inhibition of poly-ADP ribose synthetase (see Bromme & Holz, 1996; Zingarelli et al, 1996; Gilad et al, 1997; Thiememann et al, 1997).

(4) Ca^{2+} uptake inhibitors (see Ferrari et al, 1996; Loh et al, 1998; Russ et al, 1996).

(5) Addition of IPGs to established cardioplegic solutions (see Choong and Gavin, 1996; Bozkurt et al,

1997).

(6) Maintenance of glutathione systems (see Konorev et al, 1996). Glutathione in its reduced form (GSH) is an important factor in the prevention of damage by hydrogen peroxide. Hydrogen peroxide is a component of ischaemia-reperfusion injury and protection is afforded by the action of glutathione peroxidase and GSH. The importance of GSH and the pentose phosphate pathway in the chain reactions protecting the cell from free radical damage is illustrated in Figure 1 from Zubairu et al, 1983.

(7) Endothelin inhibitors (see Goodwin et al, 1997; Pernow & Wang, 1997). Endothelin-1 (ET-1) is an extremely potent vasoconstrictor peptide derived from vascular endothelial cells. During and following myocardial ischaemia and reperfusion, the myocardial production and release of ET-1 is stimulated and the coronary constriction to ET-1 is enhanced. The pathophysiological role for ET-1 in the development of ischaemia has a strong basis and the potential for cardioprotective effects of ET-1 antagonists has been considered by Pernow and Wang (1997).

Ischaemia-reperfusion injury can arise in a wide range of conditions and the medicament can be used to treat these conditions. Examples include ischaemia resulting from myocardial infarct, during surgery (especially open heart surgery, or during organ transplantation, e.g. employing the medicament as a cardioplegia solution for heart or lung bypass surgery), and in stroke. The medicament can

also be used to ameliorate the effects of ischaemia in tissues, in particular as an anti-apoptotic agent to prevent cell death following ischaemia, e.g. muscle cell death.

In a further aspect, the present invention provides a method for preserving an organ for transplantation, the method comprising exposing the organ with a composition comprising an inositolphosphoglycan (IPG), and optionally one or more of the components mentioned above. As ischaemia is common in organs for transplantation, this approach is useful for preserving the energy level present in the organ prior to transplantation and during surgery. Conveniently, the composition can be perfused through the organ or used to store the organ prior to transplantation, i.e. be a storage medium for the organ.

In a further aspect, the present invention provides compositions comprising a P-type IPG and ribose. In these compositions, the IPG drives mitochondrial oxidation and results in ATP generation from ADP without production of toxic byproducts. Preferably, the composition additionally comprises a purine or purine nucleotide precursor to provide the basic structural element of ATP. Other possible components of the composition are described above.

This composition is useful in organ preservation, in general surgery (e.g. as a perfusion fluid) and in other situations for the prevention or treatment of ischaemia in cells. Preferably, the composition is supplied as a

powder or concentrate from which a liquid composition can be prepared. Alternatively, the composition can be supplied ready to use in as a liquid. Formulations and optional ingredients of the composition are discussed further below.

In further aspects, the present invention provides above compositions for use in a method of medical treatment, for example in the preparation of a medicament for the treatment of ischaemic conditions discussed above.

Embodiments of the present invention will now be described by way of example and not by limitation with reference to the accompanying drawings.

Brief Description of the Drawings

Figure 1 shows the correlation between the hepatic PRPP concentration and the log of ribose 5-phosphate and the flux through the oxidative pentose phosphate assay pathway (C1-C6) in different dietary and hormonal conditions in rats.

Figure 2 shows the correlation between the hepatic PRPP concentration and ATP and energy charge (EC), free cytosolic NAD^+/NADH and NAD^+/NADPH in different dietary and hormonal conditions in rats.

Figure 3 shows the correlation between the hepatic PRPP concentration and ADP, AMP and Pi in different dietary and hormonal conditions in rats.

Figures 4A and 4B shows the steady state concentration and the effect of insulin on extractable IPG A-type from the heart and other tissues from adult male rats. Figure 4A shows the results of a lipogenesis assay and figure 4B shows a cAMP-dependent protein kinase A assay. The solid columns show results in the absence of insulin, while the hatched columns show results 2 minutes after injection with insulin. 1 unit is the amount of A-type IPG causing a 50% increase in the basal rate of lipogenesis or a 50% decrease in the activity of cAMP dependent protein kinase.

Figures 4C and 4D show the steady state concentration and the effect of insulin on extractable IPG P-type from heart and other tissues from adult male rats. Figure 4C shows a PDH phosphatase assay and figure 4D shows a cAMP-dependent protein kinase-P assay. The solid columns show results in the absence of insulin, while the hatched columns show results 2 minutes after injection with insulin. 1 unit is the amount of P-type IPG causing a 50% increase in the activity of PDH phosphatase or a 50% decrease in the activity of cAMP dependent protein kinase.

Figures 4E and 4F show the results of a thymidine incorporation into EGF receptor transfected 3T3 cells, plotted against IPG A-type and IPG P-type concentrations respectively.

Figure 5 shows a schematic setting out the role of ribose, IPGs and selected substrates on the prevention or

recovery from ischaemic damage according to the present invention.

Figure 6 shows a schematic setting out the site of action of IPG P-type in the activation of the PDH complex.

Detailed Description of the Invention

IPGs and IPG Analogues

Studies have shown that A-type mediators modulate the activity of a number of insulin-dependent enzymes such as cAMP dependent protein kinase (inhibits), adenylate cyclase (inhibits) and cAMP phospho-diesterases (stimulates). In contrast, P-type mediators modulate the activity of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase (stimulates), glycogen synthase phosphatase (stimulates) and cAMP dependent kinase (inhibits). The A-type mediators mimic the lipogenic activity of insulin on adipocytes, whereas the P-type mediators mimic the glycogenic activity of insulin on muscle. Both A-and P-type mediators are mitogenic when added to fibroblasts in serum free media. The ability of the mediators to stimulate fibroblast proliferation is enhanced if the cells are transfected with the EGF-receptor. A-type mediators can stimulate cell proliferation in the chick cochleovestibular ganglia.

Soluble IPG fractions having A-type and P-type activity have been obtained from a variety of animal tissues including rat tissues (liver, kidney, muscle brain, adipose, heart) and bovine liver. A- and P-type IPG biological activity has also been detected in human liver

and placenta, malaria parasitized RBC and mycobacteria. The ability of an anti-inositolglycan antibody to inhibit insulin action on human placental cytotrophoblasts and BC3H1 myocytes or bovine-derived IPG action on rat diaphragm and chick cochleovestibular ganglia suggests cross-species conservation of many structural features. However, it is important to note that although the prior art includes these reports of A- and P-type IPG activity in some biological fractions, the purification or characterisation of the agents responsible for the activity is not disclosed.

A-type substances are cyclitol-containing carbohydrates, also containing Zn^{2+} ion and optionally phosphate and having the properties of regulating lipogenic activity and inhibiting cAMP dependent protein kinase. They may also inhibit adenylate cyclase, be mitogenic when added to EGF-transfected fibroblasts in serum free medium, and stimulate lipogenesis in adipocytes.

P-type substances are cyclitol-containing carbohydrates, also containing Mn^{2+} and/or Zn^{2+} ions and optionally phosphate and having the properties of regulating glycogen metabolism and activating pyruvate dehydrogenase phosphatase. They may also stimulate the activity of glycogen synthase phosphatase, be mitogenic when added to fibroblasts in serum free medium, and stimulate pyruvate dehydrogenase phosphatase.

Methods for obtaining A-type and P-type IPGs are set out in Caro et al, 1997 and in WO98/11116 or WO98/11117. The

present invention can employ IPGs found in nature, for instance in tissues such a liver or placenta from animals such as human, pig, rat or other animals), and obtained using methods described in the above applications. These IPGs are preferably purified from the tissues, and more preferably purified to homogeneity. As defined herein, "substantially purified" describes IPGs which have been separated from components which are naturally present with the IPGs in the source tissue. Preferably, the compositions are at least 75%, more preferably at least 90%, more preferably at least 95%, and still more preferably at least 99% by weight of IPGs.

Alternatively or additionally, the present invention can employ cyclitol-containing IPG analogues, e.g. inositol-containing IPG analogues. These compounds have the advantage that they can be more readily prepared using synthetic organic chemistry methods, rather than being extracted from natural source materials. Preferred P-type synthetic analogues contain chiro-inositol, or a derivative thereof, as a structural unit or motif, and have one or more of the properties of P-type IPGs indicated above, especially activation of pyruvate dehydrogenase phosphatase. An example of a chiro-inositol containing IPG analogue is compound C4, 1D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-chiro-inositol 1-phosphate which can be synthesised as described in Jaramillo et al, 1994.

Preferred A-type synthetic analogues contain myo-inositol, or a derivative thereof, as a structural unit

or motif and have one or more of the properties of A-type IPGs indicated above. An example of a myo-inositol containing IPG analogue is compound C3 1D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-myo-inositol 1,2-(cyclic phosphate), which can be been prepared as described in Zapata et al, 1994.

Pharmaceutical Compositions

The compositions of the invention can be formulated according to the specific application which the composition is intended to treat. The compositions may comprise, in addition to the one or more IPGs, and optionally one or more of the above components, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient(s). The precise nature of the carrier or other material may depend on the route of administration, e.g. intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes. For embodiments in which the medicaments or compositions of the invention are used in organ preservation, they can be formulated so that they are suitable for storing or perfusing organs or tissue.

The compositions may be supplied in the form of a powder or concentrate from which a composition can be prepared. Alternatively, the composition may be supplied in a ready to use form, e.g. as a liquid. In either event, the composition may include other active ingredients,

adjuvants or carriers. Thus, physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

In embodiments in which the composition is used in the prophylactic or therapeutic treatment of conditions associated with a risk of ischaemia, preferably the composition is administered to a patient via intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction. In this case, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, lactated Ringer's injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. Injection is a preferred mode of delivery for compositions for treating ischaemia that results from myocardial infarction, stroke or to treat or protect against apoptosis.

The active ingredients in the composition are preferable administered to an individual in preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of

administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Experimental

Experiments in this laboratory have shown with rat heart preparations that the tissue PRPP concentration in anoxic conditions fell and was partially restored by addition of ribose to the medium. Perhaps of greater significance was our observation of the decline in cellular PRPP in a range of tissues, including heart, in experimental diabetes (see Table 1). These data suggest that ribose or a ribose precursor and/or purine derivatives could advantageously be included in the medicaments compositions of the invention.

While reported effects of repletion of cardiac ATP are encouraging, it is apparent that these biosynthetic processes themselves require ATP, as does the

reconversion of AMP to ADP and ATP, the required ATP being the very compound in short supply. Thus, any mechanism increasing the energy generating systems of the cell, primarily and most effectively via the mitochondrial oxidative restoration, would be advantageous to the process of cellular restoration. In this context, the regulation of the key enzyme for the entry of pyruvate into the tricarboxylic acid cycle, the pyruvate dehydrogenase complex, must be considered.

This enzyme is highly regulated by, among other factors, the energy status of the cell, by the NADH/NAD+ ratio and by the acetyl CoA/CoA ratio, via the interconversion of active/inactive forms of pyruvate dehydrogenase by phosphorylation/dephosphorylation reactions regulated by pyruvate dehydrogenase kinase and regulation of this enzyme complex at the pyruvate crossroads. This system operates in a manner such that ischaemic conditions activate PDH kinase dehydrogenase and so shut off energy production at this step. In order to circumvent this inhibition, even in ischaemia, it is necessary to activate the PDH phosphatase and this can be accomplished by the presence of IPGs. Pyruvate dehydrogenase activity is the most important determinant of whether pyruvate is converted to lactate, leading to lactic acidosis and a low level of ATP from glycolysis, or whether the highly efficient ATP generating system of the tricarboxylic acid cycle will be facilitated.

The present invention centres upon the use of naturally occurring activators of pyruvate dehydrogenase

phosphatase, the inositolphosphoglycans, to promote the conversion of pyruvate dehydrogenase to the active form (Rademacher et al, 1994; Varela-Nieto et al, 1998), thereby enhancing the rephosphorylation of AMP and ADP. The preferred combination of purine nucleotide precursors (to provide the basic structural element of the required ATP), together with ribose (to provide the ribose 5-phosphate for PRPP formation) and inositolphosphoglycans (to shift the pyruvate dehydrogenase complex towards the active form, generate energy and decrease lactic acidosis) can be used to treat ischaemic conditions, e.g. ischaemic heart conditions, and the loss of ATP. As can be seen from Figure 5, such a therapy would supply all three major elements required for the restoration of the energy charge of the cell.

(1) Ribose, as the precursor of the synthesis of the adenine lost from the cell during extended ischaemia;

(2) PRPP, an essential component of the adenine biosynthetic pathway; and,

(3) An increase energy yield from carbohydrate fuel which can provide the energy needed for biosynthetic processes in (1) and (2) and also to rephosphorylate such ADP and AMP as remains in the cell to ATP.

Therefore, the approach of using inositolphosphoglycans either alone or together with other precursors of adenine nucleotide synthesis and compounds protecting against loss of ATP (e.g. by inhibition of poly ADP ribose), in

the treatment of ischaemic conditions in heart, kidney, brain or other organs, is a fundamental new approach to attempting to limit cell damage. In a preferred embodiment of the invention, the combination of ribose, purine precursors and nicotinamide, the latter to prevent lost of NAD and ATP by inhibition of polyADP ribose synthase, with the inositolphosphoglycans, the potent second messenger system functioning in the regulation of protein phosphorylation/dephosphorylation cycles, is a multifaceted attack on the very basis of cellular damage in ischaemic conditions, that is the loss of ATP.

Table 1 demonstrates that in diabetes, there is a drop in tissue levels of PRPP. This drop could make diabetic patients more at risk of morbidity following an ischaemic attack. It is well established that both the incidence and complications of coronary heart disease are elevated in diabetic patients and decreased tissue levels of PRPP could be the crucial link. Thus, the present invention is particularly suited to the treatment of ischaemic conditions arising from diabetes. Figure 1 demonstrates that tissue levels of ribose 5-phosphate are important in maintaining PRPP levels and Figure 5 shows that ribose is the direct precursor of ribose 5-phosphate. Therefore, one important component in maintaining high levels of PRPP is to provide ribose as the precursor for ribose 5-phosphate.

Figures 2 and 3 demonstrate that in order to have high levels of PRPP in tissues, the cellular energy charge must be high. Under anoxic conditions, this is difficult

since the enzyme PDH kinase is activated. The action of this enzyme is to inactivate the PDH complex, which is involved in the biosynthesis of acetyl-CoA and NADH. The NADH so generated in the reperfusion period is oxidized by the electron transport chain to generate ATP. The acetyl-CoA is a substrate for the Krebs cycle in which one glucose can be oxidized to 36 ATPs via the generation of further NADH. The action of IPG-P type mediators is to activate PDH phosphatase which counteracts the PDH kinase and allows for activation of the PDH complex. This activation is shown in Figure 6. The action of the IPG-P type and the amounts recovered from various tissues before and after insulin infusion are shown in Figure 4C and D. In particular, an increase in activity is found in muscle and kidney upon insulin infusion. In contrast, decreased activity is found in heart, adipose tissue and brain (Figure 4C). These data demonstrate that an insulin infusion could not substitute for a direct infusion of the IPG-P type. Figure 5 shows that an insulin infusion will also affect the IPG-A activity differentially in tissues and this effect would not occur on infusion of just IPG-P compound or its analogues.

TABLE 1. EFFECTS OF EXPERIMENTAL DIABETES ON PHOSPHORIBOSYL PYROPHOSPHATE (PRPP) CONTENT OF HEART AND OTHER TISSUES

	PHOSPHORIBOSYL PYROPHOSPHATE CONTENT (nmoles/g tissue)		
Tissue	Control	STZ Diabetic (14 Days)	"P"
Heart	3.61±0.11 (15)	2.60±0.20 (6)	<0.01
Liver	10.5±0.64 (17)	7.60±0.43 (5)	<0.001
Lung	5.40±0.05 (16)	3.44±0.39 (5)	<0.001
Testis	5.0±0.30 (20)	2.5±0.9 (5)	<0.02
Blood glucose (mM)	7.0±0.45 (25)	28±3.0 (7)	<0.001
		226±21 (7)	<0.01
Body weight (g)	309±17 (20)		

The tissues were freeze-clamped and the PRPP content estimated as described by Kunjara et al (1987). The values are given as means ±SEM; Fisher's P values are given. The adult male rats were used 14 days after the induction of diabetes with streptozotocin.

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The following references are all expressly incorporated by reference.

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Courtesy Copy of Amended Claims for U.S. National Phase of PCT/GB99/01499

1. A composition comprising an inositolphosphoglycan (IPG) or an IPG synthetic analogue and ribose.

2. The composition of claim 1 wherein the IPG is a P-type IPG.

3. The composition of claim 1 wherein the synthetic analogue is a P-type IPG synthetic analogue.

4. The composition of claim 1, further comprising adenosine or purine, or a nucleotide precursor thereof.

5. The composition of claim 1 or claim 2, wherein the composition is a liquid composition.

6. The composition of claim 1 or claim 2, wherein the composition is a powder or concentrate from which a liquid composition can be prepared.

7. A composition of claim 1, for use in a method of medical treatment.

8. Use of an inositolphosphoglycan (IPG) or an IPG synthetic analogue for the preparation of a medicament for the treatment of an ischaemic-reperfusion injury.

9. The use of claim 8 wherein the IPG is a P-type IPG.

10. The use of claim 8 wherein the synthetic analogue is a P-type IPG synthetic analogue.

11. The use of claim 8, wherein the ischaemic-reperfusion injury arises from myocardial infarct, surgery or stroke.

12. The use of claim 11, wherein the surgery is open heart surgery, organ transplantation surgery, or heart or lung bypass surgery.

13. The use of claim 8, wherein the medicament is for the prevention of apoptosis following an ischaemic-reperfusion injury.

14. The use of claim 8 wherein the medicament further comprises one or more of:

(a) adenosine or purine or a precursor thereof;
(b) ribose;
(c) nicotinamide or derivatives thereof;
(d) a Ca^{2+} ion uptake inhibitor;

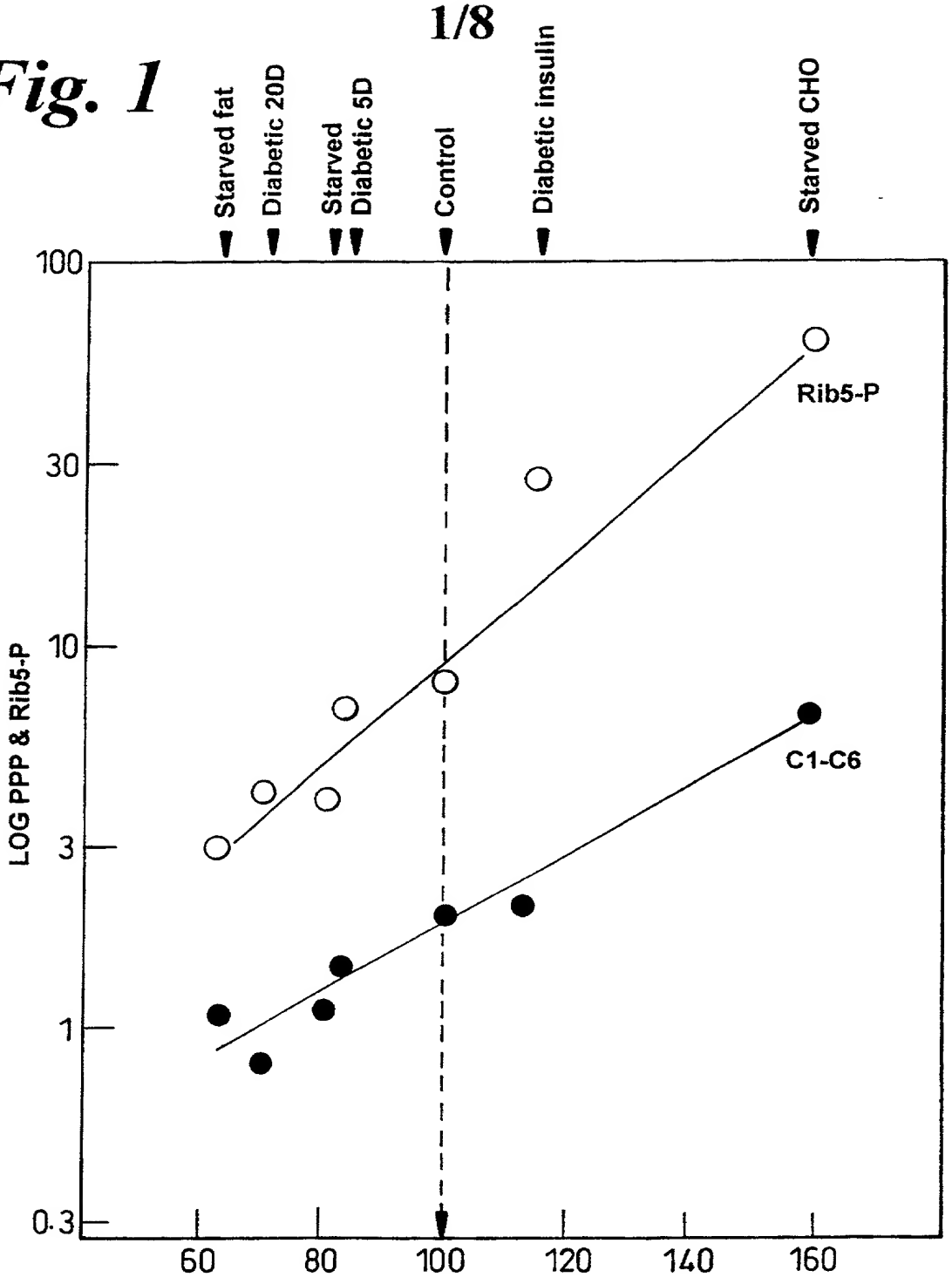
(e) a cardioplegic solution;
(f) means to maintain the glutathione system, such as glutathione peroxidase and the reduced form of glutathione (GSH); or,
(g) an endothelin inhibitor.

15. An in vitro method for preserving an organ for transplantation, the method comprising contacting the organ with a composition of claim 1.

16. The method of claim 15 wherein the composition is perfused through the organ.

17. The method of claim 15 wherein the organ is stored in the composition prior to transplantation.

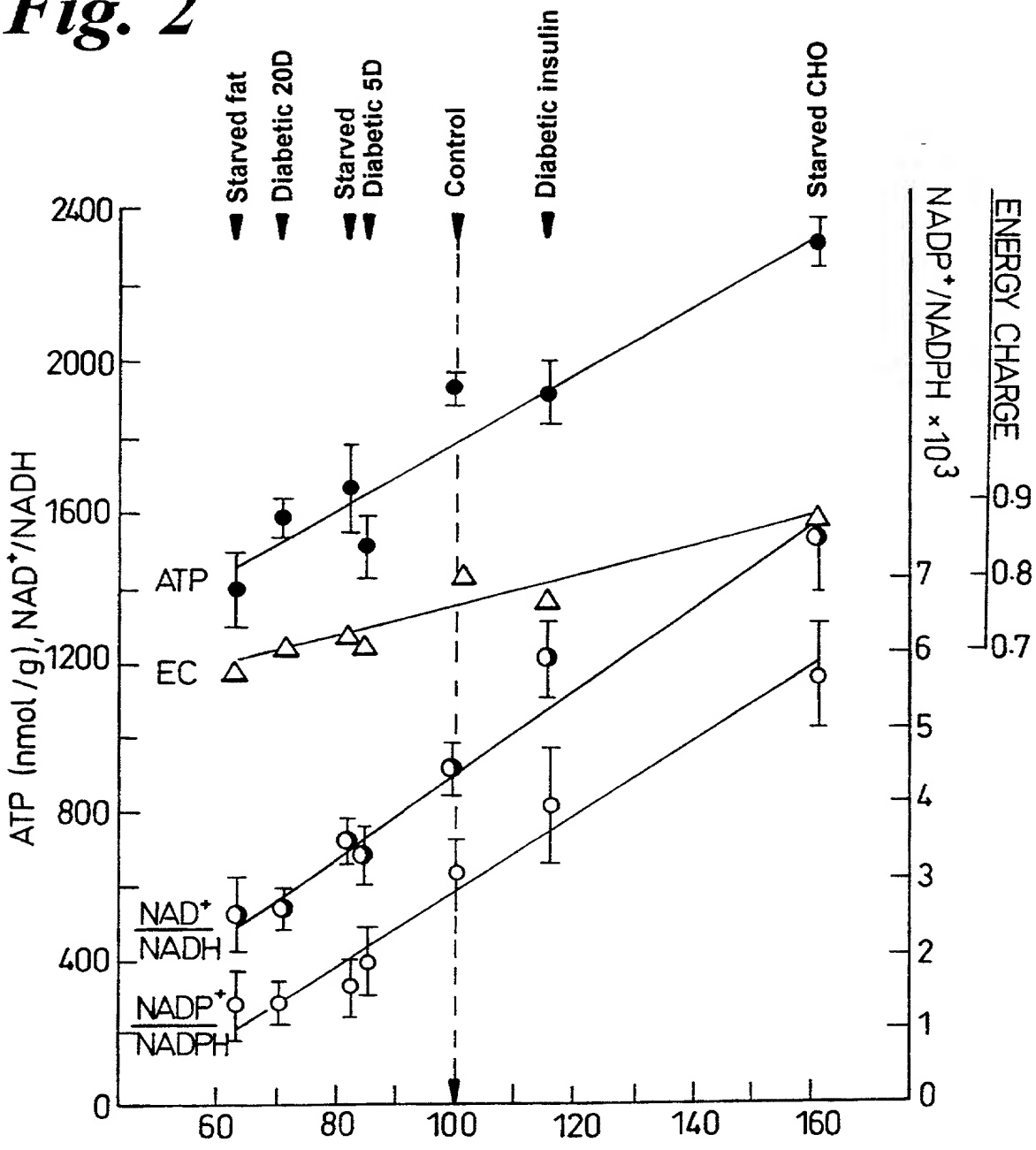
Fig. 1



PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100%

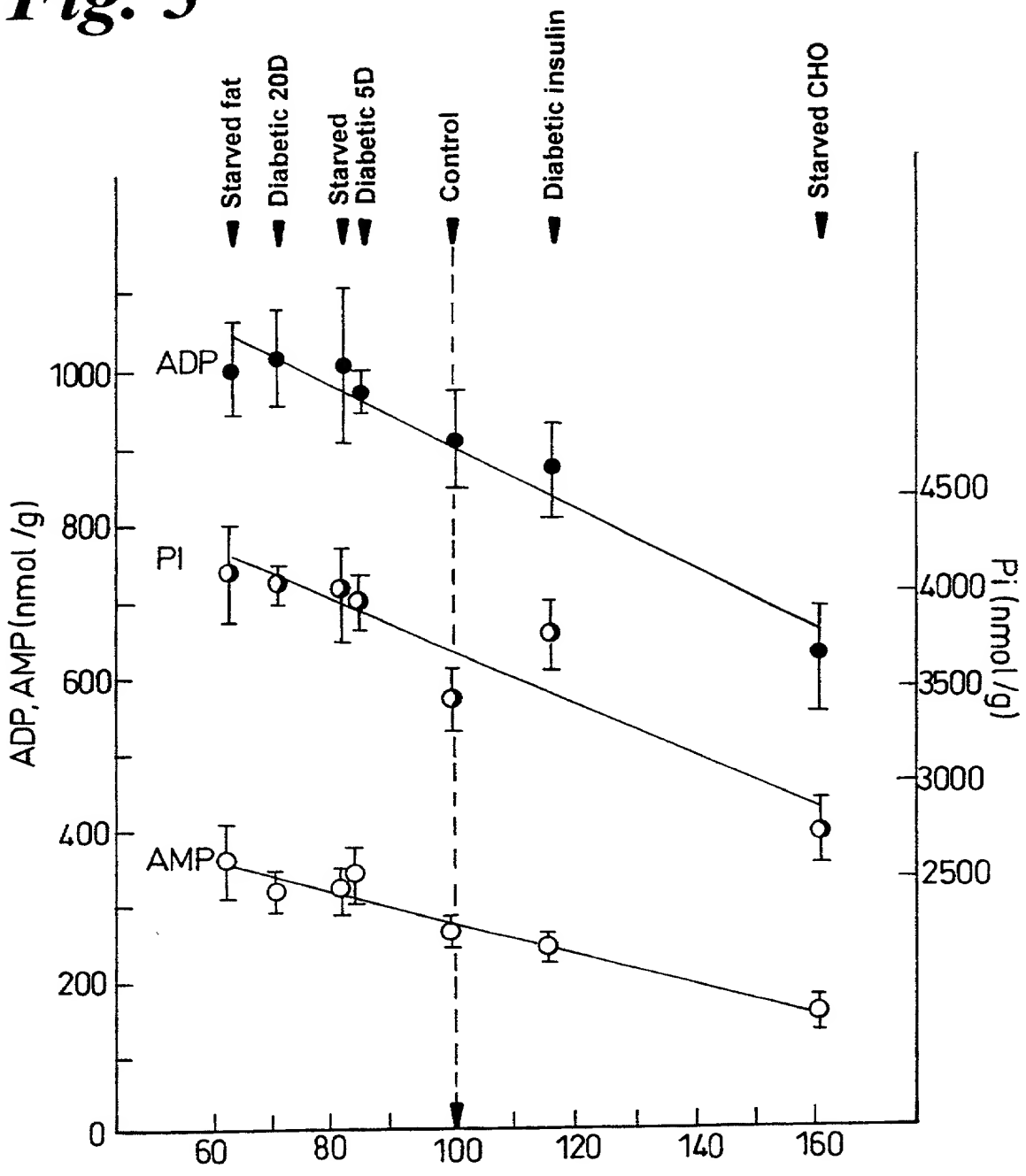
Fig. 2



PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100%

Fig. 3



PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100%

Fig. 4 (A) **Lipogenesis. Adipocyte assay.**

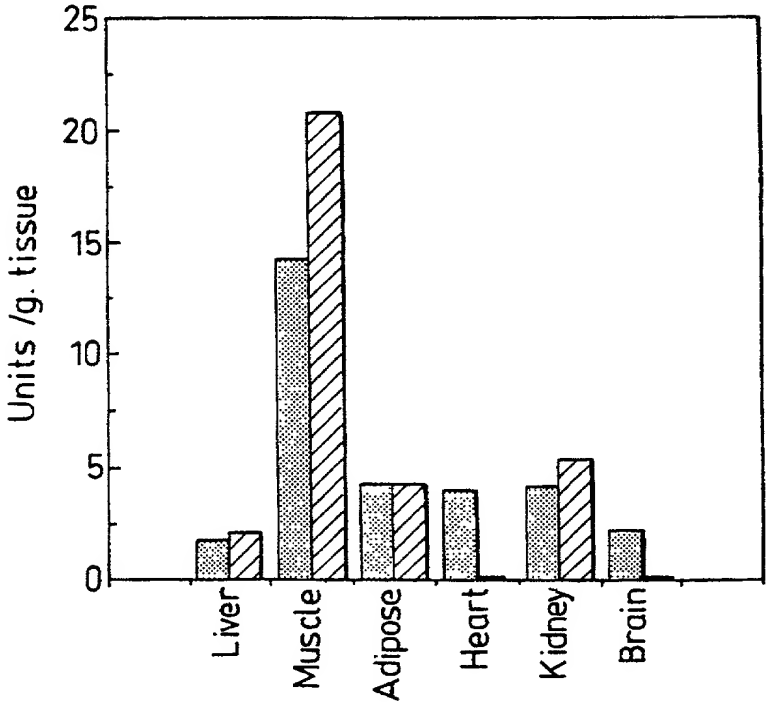


Fig. 4 (B) **cAMP-dependent protein kinase-A assay**

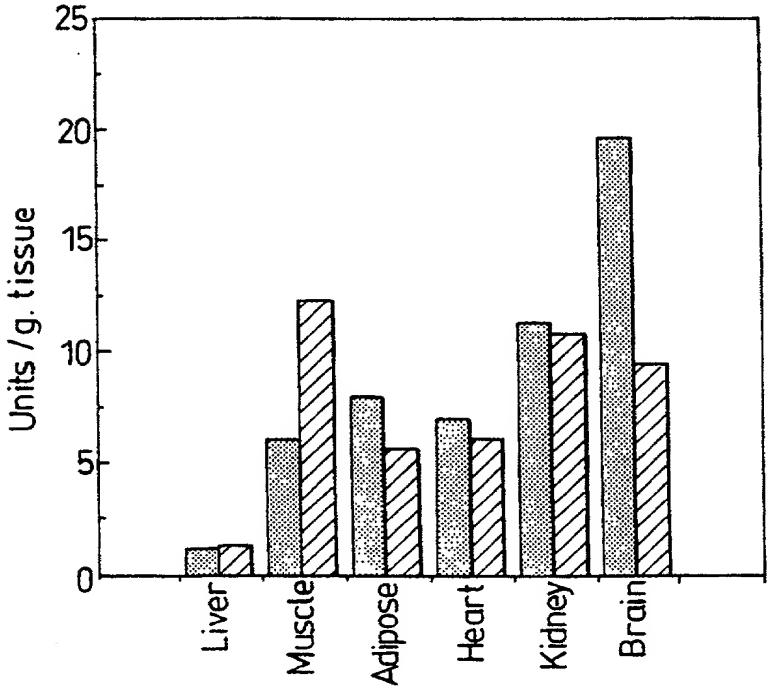


Fig. 4 (C) **PDH phosphatase assay**

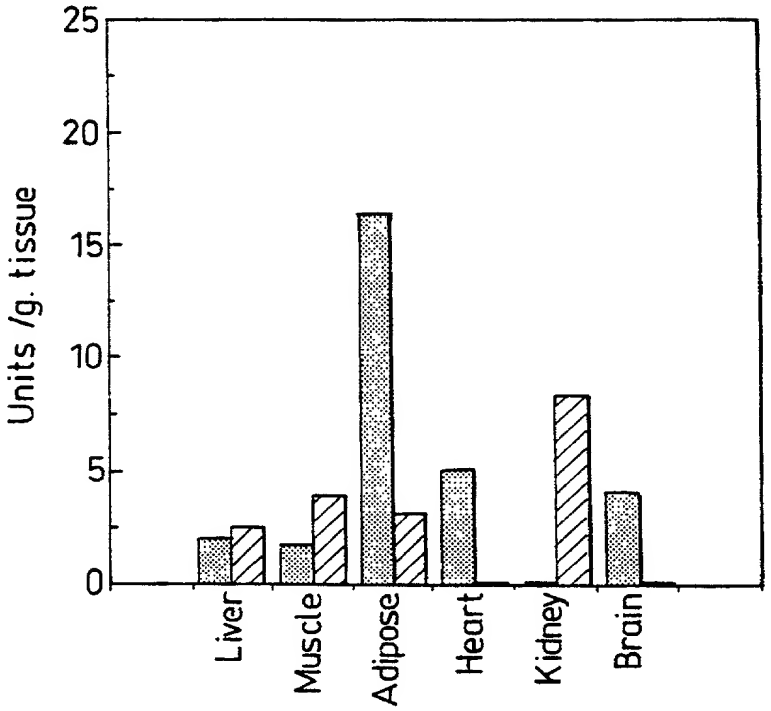
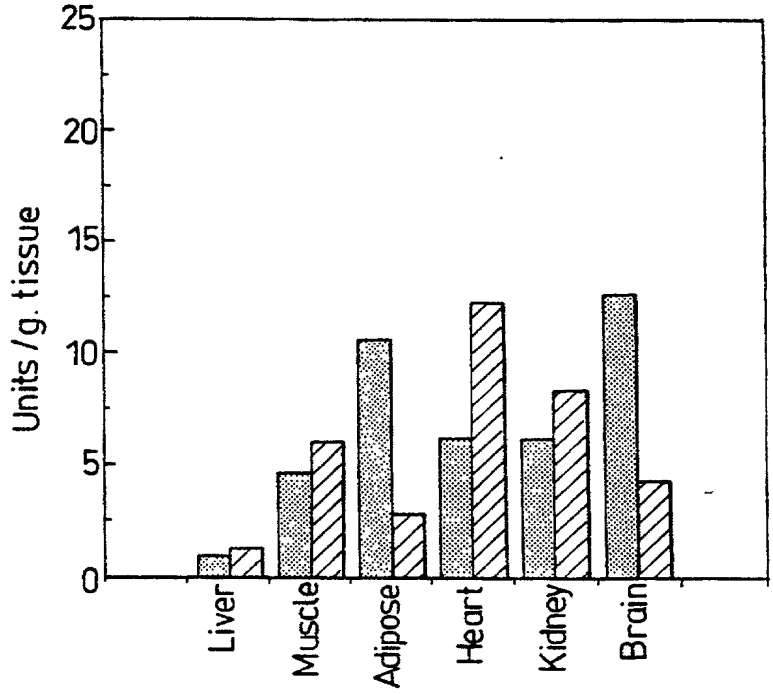


Fig. 4 (D) **cAMP-dependent protein kinase-P assay**



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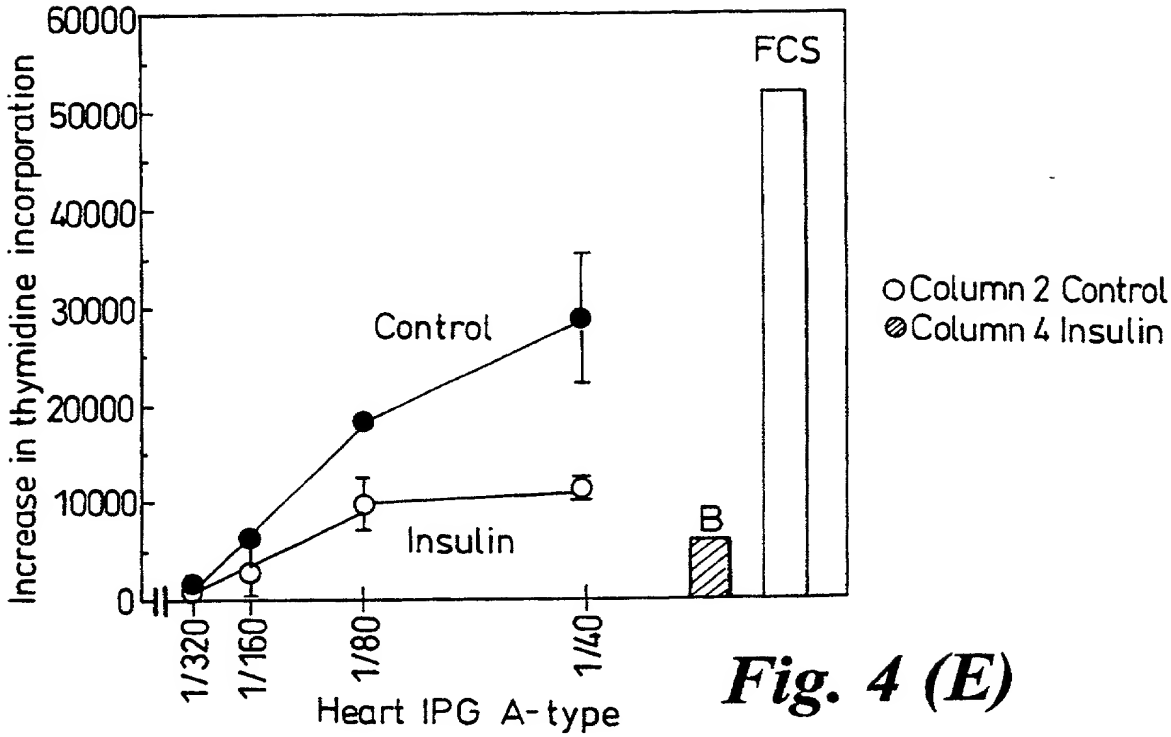

Fig. 4 (E)

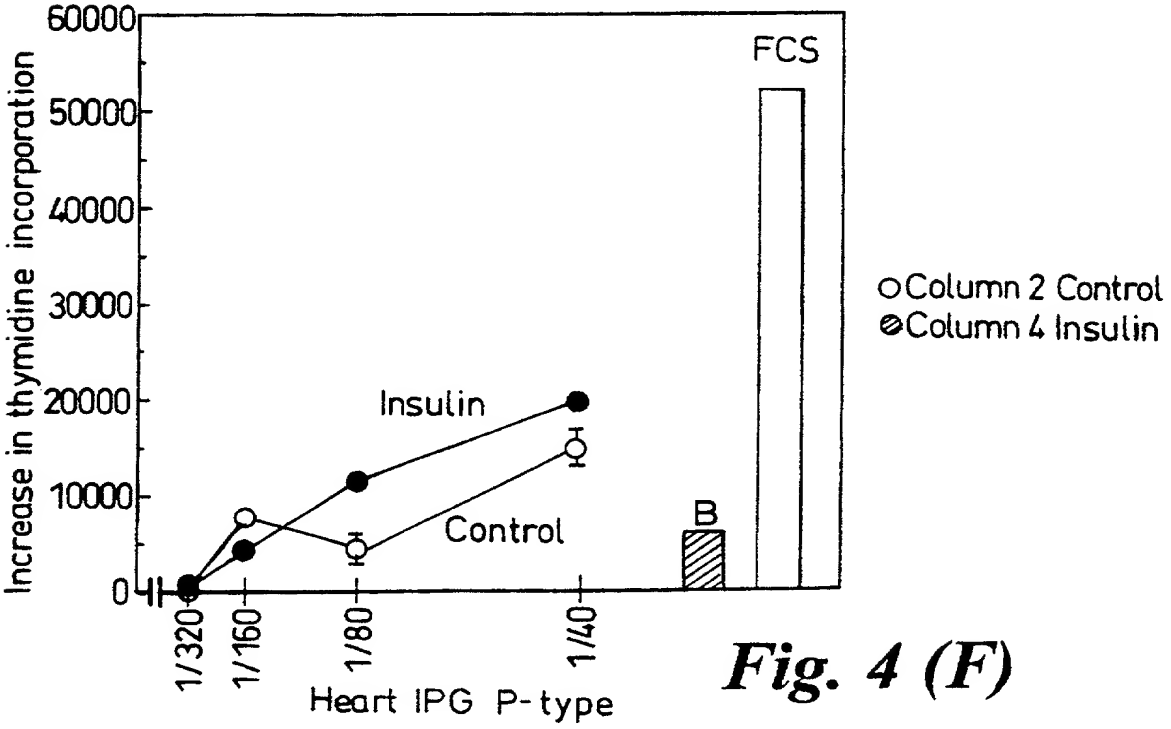

Fig. 4 (F)

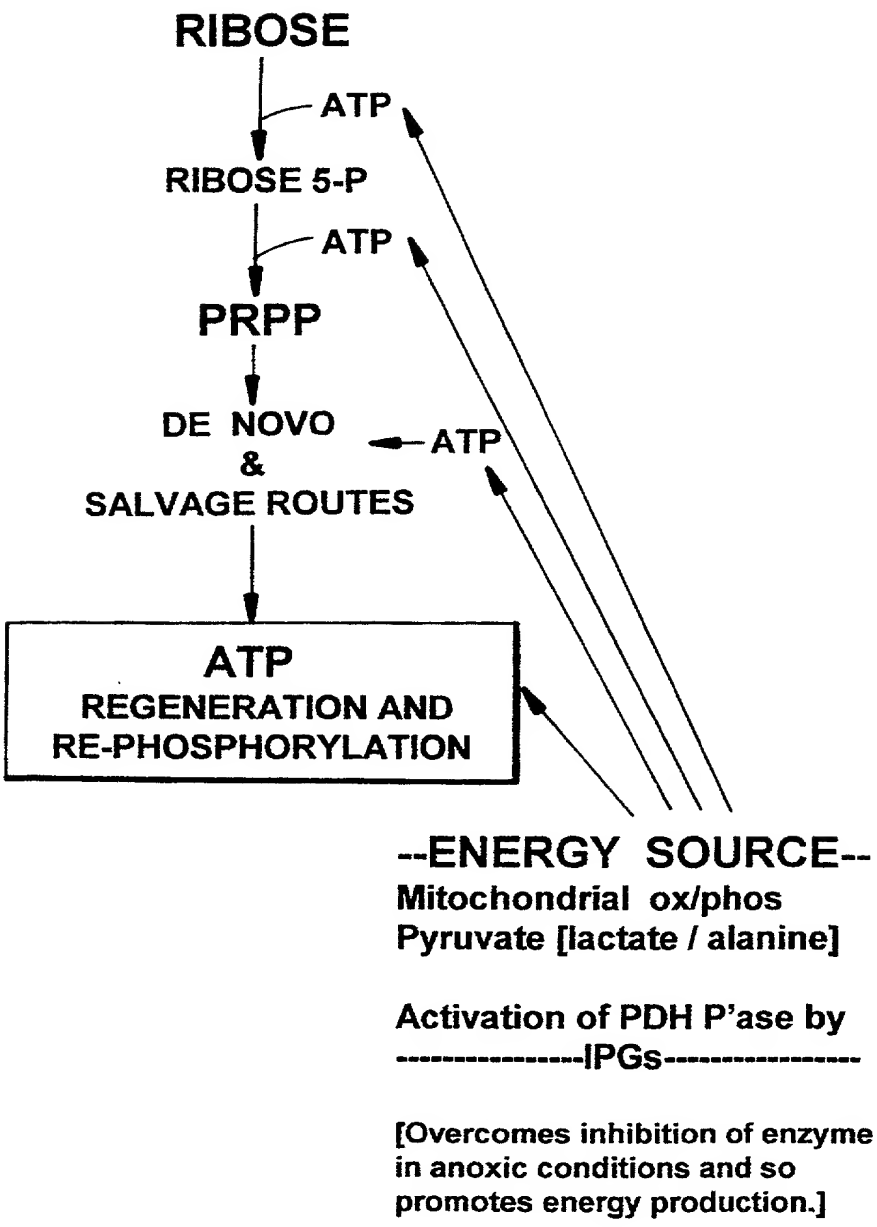
RIBOSE
ATP
RIBOSE 5-P
ATP
PRPP
DE NOVO
&
SALVAGE ROUTES
ATP
ATP
REGENERATION AND
RE-PHOSPHORYLATION
--ENERGY SOURCE--
Mitochondrial ox/phos
Pyruvate [lactate / alanine]
Activation of PDH P'ase by
----------IPGs----------
[Overcomes inhibition of enzyme
in anoxic conditions and so
promotes energy production.]

Fig. 5

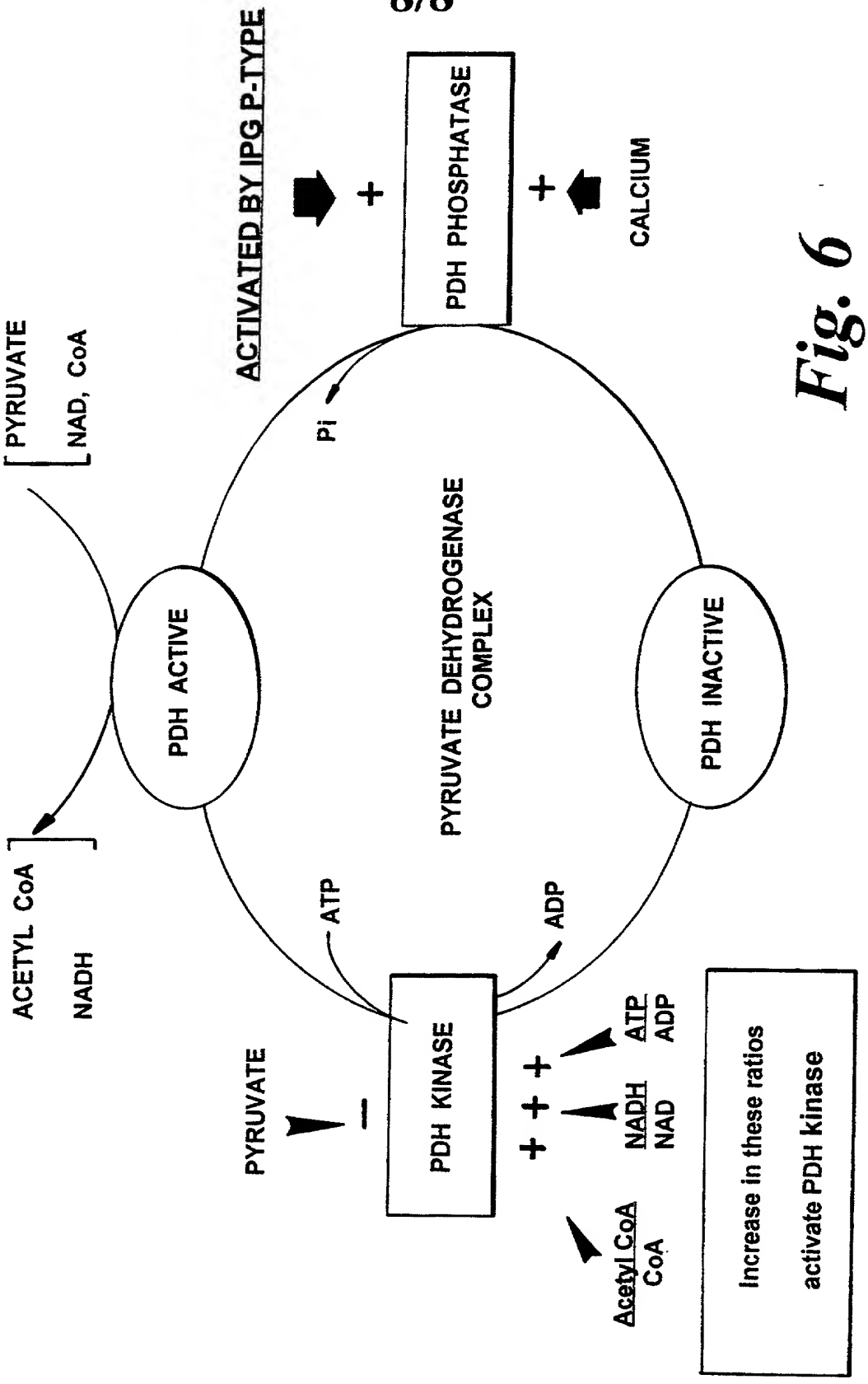
ACTIVATED BY IPG P-TYPE
PDH PHOSPHATASE
CALCIUM
PYRUVATE
NAD, CoA
Pi
PDH ACTIVE
PYRUVATE DEHYDROGENASE COMPLEX
PDH INACTIVE
ACETYL CoA
NADH
ATP
ADP
PYRUVATE
PDH KINASE
ATP
ADP
NADH
NAD
Acetyl CoA
CoA
Increase in these ratios
activate PDH kinase

Fig. 6

Attorney Docket No.: 1012-102US
Client Reference No.: **SJK/FP5891650**

DECLARATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY** the specification of which X is attached hereto or ____ was filed on as Application No. , and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
United Kingdom	9814039.5	29 June 1998	Yes

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
PCT/GB99/01499	12 May 1999	

1-00

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2-00

3-00

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Thomas Rademacher	A Greenbaum	Patricia McLean
Thomas William Rademacher	**Leslie Greenbaum**	**Patricia McLean**
Date Jan 3, 2001	Date Jan 3rd 2001	Date 3rd January 2001

declaration